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Detection of Adenovirus Hepatitis and Acute Liver Failure in Allogeneic Hematopoietic Stem Cell Transplant Patients

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Abstract

Human adenovirus (HAdV) is an important cause of the common cold and epidemic keratoconjunctivitis in immunocompetent individuals. In immunocompromised patients, HAdV can sometimes cause severe infection such as cystitis, gastroenteritis, pneumonia,

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encephalitis, hepatitis or disseminated disease, resulting in significant morbidity and also mortality. In particular, severe cases have been reported in patients after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Indeed HAdV has been recognized as a pathogen that requires careful monitoring in allo-HSCT patients. While HAdV hepatitis leading to severe acute liver failure is rare, such liver failure progresses rapidly and is often fatal. Unfortunately, HAdV hepatitis has few characteristic symptoms and physical findings, which makes it difficult to promptly confirm and start treatment. We report here four cases of HAdV hepatitis after allo-HSCT and their autopsy findings.

1. Introduction

HAdV is a double-stranded linear DNA virus that is non-enveloped and resistant to various environments and disinfectants. HAdV epidemics are common [1]. HAdV is classified into 7 species (A～G) and over 100 types by serological, genomic and bioinformatic analyses. Enteritis (mainly species F), respiratory infections (mainly species B, C, and E), pharyngoconjunctival fever, and keratoconjunctivitis (mainly species B and D) are frequent and usually mild. In contrast, disseminated disease, pneumonia, hemorrhagic cystitis (mainly species B), and hepatitis (mainly species C) are problematic and sometimes fatal in immunodeficient patients such as after organ transplantation[2]. Most of HAdVs detected in cases of HAdV infection in immunocompromised patients are C1, C2, C5, A12, A31, B3, B11, B16, B34, and B35, many of which are thought to be the reactivation of latent infectious HAdVs [3-9]. Although HAdV hepatitis is relatively rare, there are some reports of cases after allo-HSCT[10]. Despite trials on the injection of cidofovir and donor lymphocytes, most cases of HAdV hepatitis are fatal[2]. HAdV hepatitis has few characteristic symptoms and physical findings, and is often difficult to differentiate immediately from hepatic graft versus host disease (GVHD), drug-induced hepatitis, veno-occlusive disease (VOD) or sinusoidal

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obstruction syndrome (SOS), and it is not rare to delay diagnosis and treatment. A relatively rapid increase of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and biliary enzymes, imaging findings such as the appearance of a low-absorption area on liver CT, anti-HAdV antibody immunostaining by liver biopsy, virus culture identification and detection by real-time polymerase chain reaction (RT-PCR) are useful for the diagnosis of HAdV hepatitis. For a better understanding of HAdV hepatitis, we report here four patients who developed fatal HAdV hepatitis and acute liver failure after umbilical cord blood transplantation (uCBT). In all four cases, autopsy findings suggested HAdV hepatitis.

2. Case reports

Patient 1

48-year-old female was admitted to our hospital because of stomach ache and intraperitoneal lymphadenopathy, and was diagnosed with diffuse large B cell lymphoma. She achieved clinical remission with chemotherapy. Autologous hematopoietic stem cell transplantation was performed, but early recurrence occurred. Since salvage chemotherapies were ineffective, she underwent uCBT from a 5/8 major HLA-antigen (HLA-A, -B, -C, -DR antigen) matched unrelated female donor during non-remission. She received fludarabine (30 mg/m^2 once daily i.v. for 6 days), melphalan (40 mg/m^2 once daily i.v. for 2 days), and total body irradiation (4 Gy) as a conditioning regimen, and rituximab was added (375 mg/m^2 on days -8, +1 and +8). For GVHD prophylaxis, tacrolimus and mycophenolate mofetil (MMF, 1000mg/day for 4weeks) were administered. In addition, she received prophylactic acyclovir and weekly immunoglobulin. On day +9, she had systemic edema and was treated with methylprednisolone (mPSL 1mg/kg/day), furosemide, carperitide, and thrombomodulin alfa under a diagnosis of pre-engraftment syndrome. Neutrophil engraftment was achieved on day +16 and complete donor chimerism was observed on day +32. Although mPSL was

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tapered and continued in small doses, AST, ALT and biliary enzymes began to elevate from day +43. Liver acute GVHD was considered and mPSL was reincreased. Liver dysfunction and coagulation abnormalities progressed rapidly from day +52, leading to acute liver failure. Despite plasma exchange and intensive supportive care, she died of hepatic failure on day +55. RT-PCR for HAdv with her plasma from day +55 was positive (5.0×10^8copies/mL). Hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis A virus (HAV) were negative, and there were no findings of cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), or varicella zoster virus (VZV) reactivation.

Patient 2

33-year-old male was admitted to our hospital because of fever and fatigue, and was diagnosed with precursor B-acute lymphoblastic leukemia. He received peripheral blood stem cell transplantation (PBSCT) from an HLA matched sibling donor during his first complete remission. Three years after PBSCT, he had central nervous system relapse, and underwent uCBT from a 6/8 major HLA-antigen matched unrelated female donor after some salvage chemotherapies. He received fludarabine (30 mg/m^2 once daily i.v. for 5 days), melphalan (70 mg/m^2 once daily i.v. for 2 days), busulfan (3.2 mg/kg 4 times daily i.v. for 2 days), and cytarabine (3000mg/m^2 twice daily i.v. for 2 days) as a conditioning regimen. For GVHD prophylaxis, tacrolimus, MMF (1000mg/day), and rabbit antithymocyte globulin (ATG 1 mg/kg i.v. on day -1) were administered. In addition, he received prophylactic acyclovir and immunoglobulin. He had systemic edema and mild renal dysfunction from day +6 and was treated with mPSL (2mg/kg/day) under a diagnosis of pre-engraftment syndrome. mPSL was tapered and terminated on day +25. Neutrophil engraftment was achieved on day +14. On day +18, he had abnormal perception and severe pain in his limbs which were suspected to be side effects of tacrolimus. Reduction of his tacrolimus blood concentration (12→5 ng/mL)

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and an increase of MMF (2250mg/day) ameliorated his symptoms. Fever persisted and total bilirubin tended to increase from day +23, we considered GVHD. On day +37, mPSL (0.5 ㎎/kg) was resumed, fever stopped and total bilirubin decreased. MMF was gradually tapered from day +45 and was discontinued on day +87. Tacrolimus was discontinued on day +72. On day +78, CMV antigenemia turned positive and AST, ALT and biliary enzymes were elevated. These were resolved by ganciclovir administration. From day +97, fever, re-elevation of hepatobiliary enzymes, and pancytopenia advanced. On day +104, abdomen computed tomography revealed numerous low-absorption areas in the liver (Fig. 1 (a)). On day +105, platelet transfusion was performed (35 units in total), and percutaneous liver biopsy was performed on day +106. Hematoxylin and eosin staining of the liver biopsy tissue showed widespread and patchy necrosis with minimal inflammatory cell infiltration. No tumor infiltration was observed. Eosinophilic nuclear inclusions were found in hepatocytes around the necrotic tissue and the hepatocytes were immunohistochemically positive for HAdV. Peritoneal hemorrhage persisted after liver biopsy, the level of consciousness rapidly deteriorated on day +109, and he died (suspected of cerebral hemorrhage). HAdV and CMV were both positive by RT-PCR on his plasma from day +106 (1.97×10^9 copies/mL and 1.95×10^4 copies/mL, respectively). HBV, HCV and HAV were negative, and there were no findings of EBV, HHV-6, or VZV reactivation.

Patient 3

51-year-old male was admitted to our hospital because of systemic lymphadenopathy and was diagnosed with adult T-cell leukemia-lymphoma. He achieved complete remission with chemotherapy and mogamurizumab. CMV reactivation occurred several times during treatment. He underwent uCBT from a 3/8 major HLA-antigen matched unrelated male donor. He received fludarabine (30 mg/m^2 once daily i.v. for 6 days), melphalan (40 mg/m^2 once

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daily i.v. for 2 days), and busulfan (3.2 mg/kg 4 times daily i.v. for for 2 days) as a conditioning regimen. For GVHD prophylaxis, tacrolimus and ATG (1 mg/kg i.v. on day -1) were administered. In addition, he received prophylactic valaciclovir. He had hemorrhagic cystitis on day +11, and this resolved in 10 days. Neutrophil engraftment was achieved on day +14 and complete donor chimerism was noted on day +78. On day +19, CMV antigenemia turned positive and ganciclovir was administered. He exhibited nausea, pruritus and systemic edema from day +21 and was treated with mPSL (20mg/day) under a diagnosis of engraftment syndrome. mPSL was tapered and continued in small doses. On day +27, plasma PCR revealed that CMV was positive (HHV-6 was negative and HAdV was not measured). On day +26, severe lower limb pain appeared. The possibility of Calcineurin-inhibitor Induced Pain Syndrome was considered, and we switched tacrolimus to MMF (1500mg/day). His pain was reduced and MMF was discontinued on day +86. Beginning on day +90, memory and cognitive disorder, convulsion and fever appeared, and no abnormal findings were found on head MRI. Thrombotic microangiopathy (TMA) was considered and he received corticosteroid pulse treatment and plasma exchange (3 consecutive days), but his symptoms did not diminish. On day +95, MRI revealed abnormal high signal around the right ventricle on T2 and FLAIR, and RT-PCR of his cerebrospinal fluid was positive for CMV (2.26×10^4 copies/mL). CMV encephalitis was considered and ganciclovir and foscarnet were administered simultaneously, but were ineffective. On day +101, he received corticosteroid pulse again; he regained consciousness and was able to respond. Beginning on day +110, red blood cell fragmentation appeared, renal dysfunction progressed, lactate dehydrogenase was elevated, and consciousness disorder relapsed. He received continuous hemodiafiltration and plasma exchange under a diagnosis of TMA relapse, but they were not effective. RT-PCR of his cerebrospinal fluid from day +114 was still positive for CMV (3.6×10^4 copies/mL). Beginning on day +130, AST, ALT and biliary enzymes were dramatically

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elevated, and led to acute liver failure. He died of multiple organ failure on day +136.

Patient 4

51-year-old male was admitted to our hospital because of fever and was diagnosed with FMS-like tyrosine kinase 3 internal tandem duplication (FLT3/ITD) mutation-positive acute myeloid leukemia. Chemotherapies were ineffective, and remission could not be achieved. He received allo-HSCT four times in seven months and early relapse occurred repeatedly (1st:uCBT, 2nd and 3rd:HLA-haploidentical peripheral blood stem cell transplantation, 4th:uCBT). Relapse of the 1st allo-HSCT was confirmed on day +62, relapse of the 2nd was noted on day +37 and relapse of the 3rd occurred on day +37. As 4th allo-HSCT, he underwent uCBT from a 5/8 major HLA-antigen matched unrelated male donor. He received fludarabine (30 mg/m^2 once daily i.v. for 6 days), melphalan (50 mg/m^2 once daily i.v. for 2 days) and busulfan (3.2 mg/kg 4 times daily i.v. for 2 days) as a conditioning regimen. For GVHD prophylaxis, tacrolimus was administered. He had acute renal failure and anuria on day 0, and received continuous hemodiafiltration. Subcutaneous hematoma and oral bleeding appeared due to a severe bleeding tendency. For airway management, he needed tracheal intubation and ventilation. Neutrophil engraftment was achieved on day +14. Total bilirubin increased gradually after uCBT and it exceeded 20 mg/dL on day +19. mPSL (0.5 mg/kg) was added under a diagnosis of TMA or acute liver GVHD. From day +27, AST, ALT and biliary enzymes dramatically increased, respiratory circulation became unstable, and multiple organ failure progressed. He died on day +29.

All 4 cases met the published criteria of acute liver failure[11].

Necropsy was performed with the consent of the family in all 4 cases. Common findings

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included slight hepatomegaly and increased liver weight by about 1500-1600 g. Many nodular necrotic lesions of about 1 ～ 10 mm were found in the liver (Fig. 1 (b)(c)(d)). Hematoxylin and eosin staining of the liver showed patchy necrosis with minimal inflammatory cell infiltration. Hepatocytes had enlarged glassy nuclei and intranuclear inclusions, or a 'smudged' appearance (Fig. 1 (e)(f)). In all 4 cases, the hepatocytes were immunohistochemically positive for HAdV (Fig. 1 (g)(h)) and negative for CMV (Supplemental Figure (a)(b)(c)(d)). Nested PCR analysis with extracted DNA from the liver tissue showed no significant gene replication of HHV-6.

Analysis of the liver tissue in Case 1 with the Basic Local Alignment Search Tool revealed that the HAdV was species C and type 6. In Case 2, 3 and 4, sequence analysis of HAdV obtained from plasma revealed that HAdV was species C and type 1, 1, and 5, respectively (99%, 100%, and 99% coincidence with the registered strains in the hexon region, respectively).

Discussion

The incidence of HAdV infection ranges between 2% and 15% in allo-HSCT patients, in whom it manifests as cystitis, gastroenteritis, pneumonia, encephalitis, hepatitis or disseminated systemic infection, and is frequently encountered after Herpes simplex virus and CMV[12-16]. Previous reports have shown that the risk factors for post allo-HSCT HAdV infection include GVHD of grade III or IV, detection of HAdV at two or more sites, T cell-depleted grafts, unrelated donors, cord blood transplants, haplo transplants, Alemtuzumab and ATG administration. Three of our 4 cases were re-transplant cases after auto- or allo-HSCT and achieved poor tumor control, which might have caused severe immunodeficiency and led to the onset of HAdV hepatitis [17-25]. All 4 cases required mPSL due to suspected GVHD or TMA a few days before transaminase and biliary enzymes were dramatically

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elevated, which might provoke HAdV hepatitis. Although HAdV hepatitis after allo-HSCT is rare, the mortality rate is very high, similar to those of HAdV pneumonia and disseminated infection.

Our cases were caused by species C (types 1, 5 and 6). Although there is no specific examination for HAdV hepatitis, the detection of HAdV detection in blood samples (RT-PCR of plasma or viral culture) and the appearance of an intrahepatic low-absorption area on CT are helpful for an early diagnosis. It has been reported that the HAdV copy number in PCR on plasma of hepatitis patients is drastically elevated, and turns positive several weeks before the onset of hepatitis[10, 26]. In addition, an increase in γGTP (100 IU/L or more) appears more than 2 weeks before the onset of hepatitis, suggesting that it may be useful for the early diagnosis of HAdV hepatitis[10]. In immunocompromised patients, early treatment should be considered if plasma HAdV turns positive to prevent progression to a fatal HAdV infection. Besides HAdV hepatitis, the etiology and pathogenesis of post allo-HSCT liver dysfunction include CMV hepatitis, HHV-6 hepatitis, hepatic GVHD, drug-induced hepatitis and VOD/SOS[27, 28]. For a differential diagnosis, liver biopsy should be considered, but percutaneous liver biopsy carries a risk of lethal bleeding, as in Case 2. When biopsy and histology are indispensable because it is difficult to distinguish from other causes, transvenous or transportal liver biopsy should be considered to prevent lethal bleeding[29]. However, since transvenous liver biopsy has been reported to cause bleeding from approached vessels, caution is required [30].

We experienced 4 cases of acute lethal liver failure after allo-HSCT. When misidentified as other etiology and pathogenesis of post allo-HSCT liver dysfunction, it is possible that HAdV hepatitis, which could have been overlooked because detailed HAdV examination was not performed, was present in patients who died of liver dysfunction or liver failure after allo-HSCT. HAdV hepatitis should be considered when there is a dramatic increase in liver test

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values or rapid liver failure. As with CMV, EBV, and HHV-6, the presence of HAdV should be periodically checked by RT-PCR. Some reports have suggested that HAdV in plasma should be monitored once a week for allo-HSCT recipients with one or more risk factors until adequate immune reconstitution is achieved[22, 31]. HAdV hepatitis, unlike pneumonia and cystitis, has poor subjective symptoms, and diagnosis and treatment tend to be delayed. The present study underscores the importance of the PCR diagnosis of HAdV after allo-HSCT. Although cidofovir, ribavirin, reduction of immunosuppressive treatment, and donor lymphocyte infusion have been used for HAdV infection, there is no established treatment method, and the mortality of severe cases is high (Table2). The side effect profile of cidofovir includes nausea, myelotoxicity and severe nephrotoxicity. Brincidofovir, an orally bioavailable lipid-conjugate of the nucleotide analog cidofovir, has relatively mild side effects and is considered a promising therapeutic agent for HAdV infection. Although brincidofovir can cause diarrhea, is not associated with severe renal tubulopathies and myelosuppression[32, 33]. Even though administration of cidofovir or brincidofovir may be accompanied by several side effects, when HAdV hepatitis is suspected, empiric treatment with these medications should be started as soon as possible to prevent fatal liver failure.. HAdV hepatitis is rare, but should be considered as a potential cause of acute liver failure after allo-HSCT.

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Acknowledgements

Authors Contribution

Yoshiyuki Onda: Writing – Original Draft, Writing – Review & Editing, Investigation and Data Curation. Junya Kanda: Writing – Original Draft and Conceptualization. Soichiro Sakamoto: Investigation. Mutsumi Okada: Investigation. Naoyuki Anzai: Investigation and Conceptualization. Hiroshi Umadome: Investigation. Masaro Tashima Investigation. Hironori Haga: Investigation and histopathological analysis. Chihiro Watanabe: Investigation and histopathological analysis. Nozomu Hanaoka: Investigation and viral analysis. Tsuguto Fujimoto: Investigation and viral analysis. Akifumi Takaori-Kondo: Supervision and Project administration.

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This work was supported in part by Fujiwara Memorial Foundation (J.K.).

Table 1

	Age, Sex	Disease	HLA	Stem cell	Conditioning regimen	GVHD prophylaxis	Acute GVHD	Steroid	HAdV hepatitis onset day	HAdV RT-PCR (plasma) (copies/mL)	Death day	peak value AST (IU/L)	ALT (IU/L)	LDH (IU/L)	ALP (IU/L)	γGTP (IU/L)	T-Bil (mg/dL)	CRE (mg/dL)	Immunohistochemical stain of the liver tissue HAdV	CMV	Nested PCR with extracted DNA from the liver tissue HHV-6	HAdV type
Case 1	48, F	DLBCL	Mismatched 5/8	CB	FLU+MEL+TBI(4Gy)+RIT	Tacrolimus, MMF	-	+	43	5.0×10^{8} (day55)	55	12225	2552	17032	2444	760	2.2	1.4	+	-	-	6
Case 2	33, M	B-ALL	Mismatched 6/8	CB	FLU+MEL+BU+AraC	Tacrolimus, MMF, ATG	-	+	97	1.97×10^{9} (day106)	109	4752	2007	5554	1024	832	3.3	3.2	+	-	-	1
Case 3	51, M	ATL	Mismatched 3/8	CB	FLU+MEL+BU	Tacrolimus, ATG	-	+	130	n/a	136	11268	2207	28087	2802	1241	8.3	5.1	+	-	-	1
Case 4	47, M	AML	Mismatched 5/8	CB	FLU+MEL+BU+GO	Tacrolimus	-	+	23	n/a	29	2086	254	4151	860	178	31.3	4.3	+	-	-	5

Abbreviations: DLBCL, diffuse large B-cell lymphoma; ALL, acute lymphoblastic leukemia; ATL, adult T-cell leukemia; AML, acute myeloid leukemia; HLA, human leukocyte antigen; CB, cord blood; FLU, fludarabine; MEL, melphalan; TBI, total body Irradiation; RIT, rituximab; BU, busulfan; AraC, cytarabine; GO, gemtuzumab ozogamicin; GVHD, graft versus host disease; MMF, mycophenolate mofetil; ATG, Anti thymocyte globulin; HAdV, human adenovirus; RT-PCR, real-time polymerase chain reaction; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase;

1 γGTP, γ-glutamyl transpeptidase; T-Bil, total bilirubin; CRE, creatinine; CMV, cytomegalovirus; HHV-6, human herpesvirus 6; n/a, not available.
2

1 Table 2

References	Age,Sex	Disease	Sero-type	HAdV detection					Donor	HLA	Stem cell	T-cell depletion	Acute GVHD	Steroid	Treatment	Survival
				PCR		Viral culture positive cites	IHC(Liver)	EM(Liver)								
				Blood	Liver											
Shields, 1985[34]	24, F	AML	1	n/a	n/a	Liver	+	n/a	n/a	n/a	BM	n/a	+	n/a	-	Dead
	13, M	AA	5	n/a	n/a	Liver	+	n/a	n/a	n/a	BM	n/a	+	n/a	-	Dead
Purtilo, 1985[35]	19, M	X-linked LP	5	n/a	n/a	Liver	n/a	n/a	R	Matched	BM	n/a	+	+	-	Dead
Johnson, 1990[36]	34, M	NHL	5	n/a	n/a	Urine	n/a	+	R	Matched	BM	-	+	+	-	Dead

Niemann, 1993[37]	9, F	AML	5, 12	n/a	n/a	Liver, stool, urine	n/a	+	R	Mismatched	BM	ex vivo, ATG	-	+	-	Dead
Flomenberg, 1994[38]	2, n/a	ALL	1	n/a	n/a	Liver, blood, urine, colon	n/a	n/a	R	Matched	BM	ex vivo	+	+	-	Dead
	30, n/a	CML	1	n/a	n/a	Liver, stool, urine	n/a	n/a	UR	n/a	BM	ex vivo	+	+	-	Dead
	41, n/a	CML	35	n/a	n/a	Stool, urine	n/a	n/a	R	Mismatched	BM	ex vivo	-	+	-	Dead
Charles, 1995[39]	0.7, M	Infantile OP	32	n/a	n/a	Stool, small intestine	+	n/a	n/a	n/a	BM	Alemtuzumab	n/a	n/a	-	Dead
Bertheau, 1996[40]	22, M	CML	2	n/a	n/a	Blood, stool, colon	+	+	R	Matched	BM	-	+	+	-	Dead
Chakrabarti, 1999[41]	44, M	CML	2	+	n/a	Liner, stool	n/a	+	UR	Matched	BM	Alemtuzumab	+	+	Rivavirin	Dead

Hale, 1999[42]	3, M	AML	n/a	n/a	n/a	Blood	n/a	n/a	R	Matched	BM	-	-	n/a	-	Dead
	24, F	ALL	n/a	n/a	n/a	Liver, blood, urine	n/a	n/a	UR	Matched	BM	ex vivo	-	n/a	-	Dead
	3, F	AML	5, 11	n/a	n/a	Stool, urine	n/a	n/a	R	Mismatched	BM	ex vivo	+	n/a	-	Dead
	3, M	CML	n/a	n/a	n/a	Stool	n/a	n/a	R	Mismatched	BM	ex vivo	+	n/a	-	Alive
Somervaille, 1999[43]	35, F	HL		2	n/a	Liver	+	+	R	n/a	BM	ex vivo, Alemtuzumab	-	-	DLI	Dead
Chakrabarti, 2002[12]	22, F	n/a		2	+	n/a	Stool	n/a	n/a	R	Matched	n/a	Alemtuzumab	-	-	Rivavirin	Dead
	43, M	n/a		2	+	n/a	Stool, urine	n/a	n/a	UR	Matched	n/a	Alemtuzumab	+	+	Rivavirin	Dead

Wang, 2003[44]	21, M	ALL	n/a	n/a	n/a	Liver, blood	+	n/a	n/a	n/a	BM	n/a	+	n/a	-	Dead
Nakazawa, 2006[45]	51, F	ALL	n/a	+	n/a	n/a	+	+	UR	Matched	BM	-	+	+	-	Dead
Neofytos, 2007[46]	23, M	ALL	n/a	+	n/a	Liver, stool	n/a	n/a	R	Matched	n/a	ex vivo, ATG	-	-	DLI, Cidofovir	Alive
	39, M	ALL	n/a	+	n/a	Stool, urine, colon	n/a	n/a	R	Mismatched	n/a	ex vivo, ATG	-	-	DLI, Cidofovir	Dead
	43, M	AML	n/a	+	n/a	Liver, stool	n/a	n/a	R	Matched	n/a	ex vivo, ATG	+	+	DLI, Cidofovir	Alive
	72, M	AML	n/a	+	n/a	colon	n/a	n/a	UR	Matched	n/a	-	+	+	Cidofovir	Dead
Kalpoe, 2007[47]	60, M	CLL	1	+	n/a	Stool, intestine	n/a	n/a	R	Matched	n/a	ex vivo	-	n/a	Cidofovir	Dead

Willems, 2008[48]	26, M	ALL/AA	C		+	n/a	n/a	n/a	R	Matched	PBSC	ATG	-	-	Cidofovir	Alive
Forstmeyer, 2008[49]	39, M	NHL		2	+	+	n/a	n/a	+	UR	Matched	PBSC	ATG	+	+	-	Dead
Terasako, 2012[50]	58, F	AA	n/a		+	n/a	n/a	+	n/a	UR	Matched	BM	ATG	+	+	-	Dead
Vyas, 2012[51]	46, M	ALL		5	n/a	n/a	n/a	+	n/a	UR	Matched	BM	ex vivo	+	+	-	Dead
	38, F	NHL		2	n/a	n/a	Liver, blood	+	n/a	UR	Matched	BM	-	+	+	Ribavirin	Dead
Kawashima, 2015[10]	13, F	AML		2	+	n/a	Blood, urine	+	+	R	Mismatched	BM	ATG	+	+	Cidofovir, DLI	Dead
	16, F	AA		2	+	n/a	Blood, urine	n/a	n/a	UR	Matched	BM	ATG	-	+	Cidofovir	Dead
Lo, 2015[52]	24, F	Crohn's disease	n/a		+	n/a	n/a	+	n/a	UR	n/a	CB	n/a	n/a	n/a	-	Dead

Detrait, 2015[30]	27, F	AML		+	n/a	n/a	+	n/a	R	Mismatched	PBSC	ATG	-	+	-	Dead
Schaberg, 2017[53]	47, n/a	n/a	A	+	+	Blood, stool, urine	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	Dead
	22, n/a	n/a	A, B	+	n/a	Blood, stool, urine	+	n/a	n/a	n/a	n/a	n/a	n/a	n/a	-	Dead
Present report	48, F	NHL	6	+	+	n/a	+	n/a	UR	Mismatched	CB	-	-	+	-	Dead
	33, M	ALL	1	+	+	n/a	+	n/a	UR	Mismatched	CB	ATG	-	+	-	Dead
	51, M	ATL	1	n/a	+	n/a	+	n/a	UR	Mismatched	CB	ATG	-	+	-	Dead
	47, M	AML	5	n/a	+	n/a	+	n/a	UR	Mismatched	CB	-	-	+	-	Dead

Abbreviations: AML, acute myeloid leukemia; AA, aplastic anemia; LP, lymphoproliferative disease; NHL, non-Hodgkin lymphoma; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; OP, osteopetrosis; HL, Hodgkin lymphoma; PCR, polymerase chain reaction; IHC, immunohistochemistry; EM, electron microscopy; R, related donor; UR, unrelated donor; BM, bone marrow ; PBSC, peripheral blood stem cell; CB, cord blood; ATG, Anti thymocyte globulin; GVHD, graft versus host disease; DLI, donor Lymphocyte Infusion; n/a, not available.

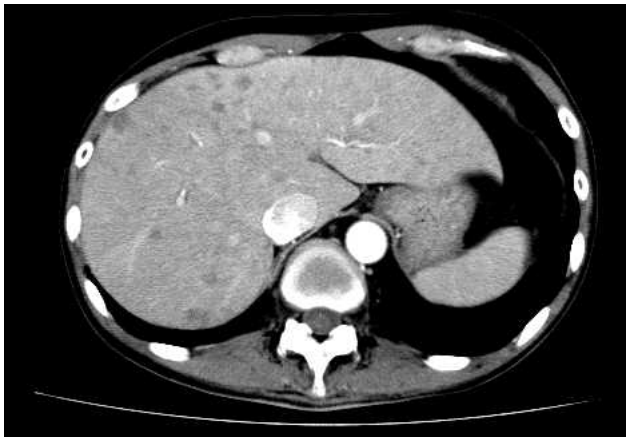
Figure legend

Figure 1: Radiological and histological presentation of adenoviral hepatitis.

(a) Enhanced CT of the abdomen revealed multiple hypodense lesions in the liver (Case2 day104). (b) appearance of the liver (Case1). (c)(d) split face of the liver (c=Case1, d=Case2). (e)(f) Hematoxylin–eosin (H&E) staining of necrotic hepatocytes (Case 4). (g)(h) Immunohistochemical staining for adenovirus (Case 2).

Figure 1

(a)



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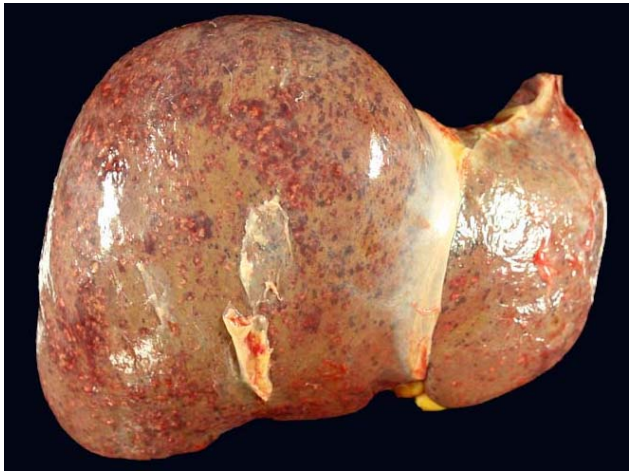

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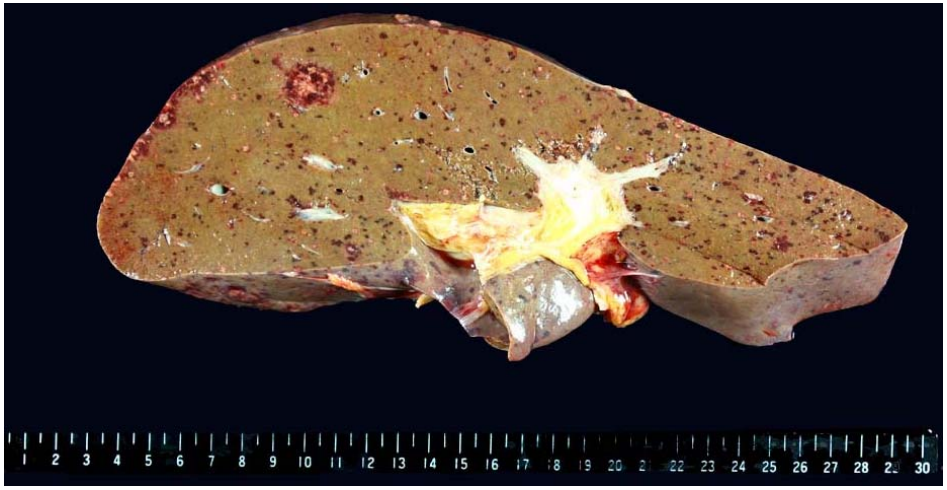

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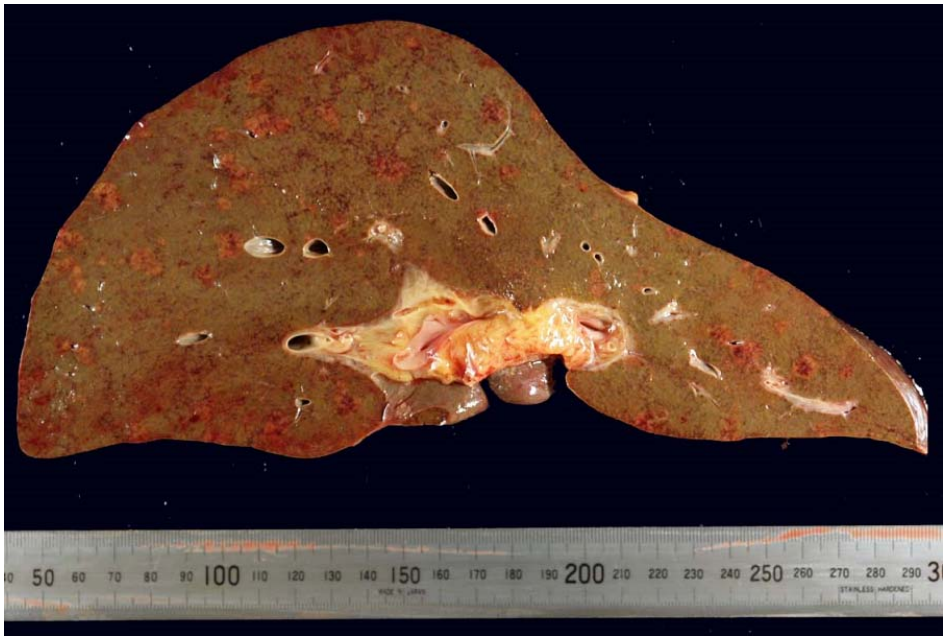

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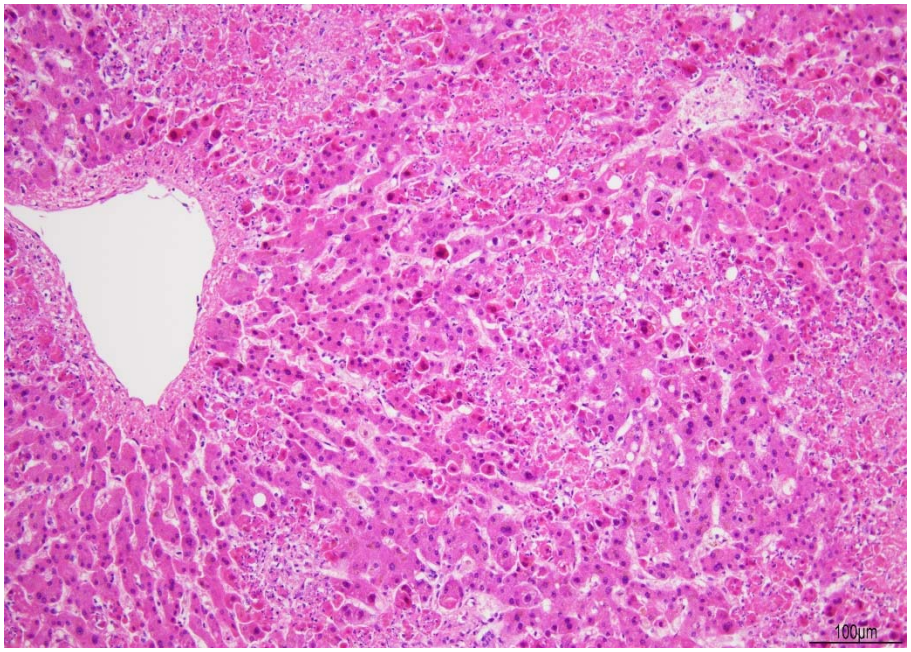

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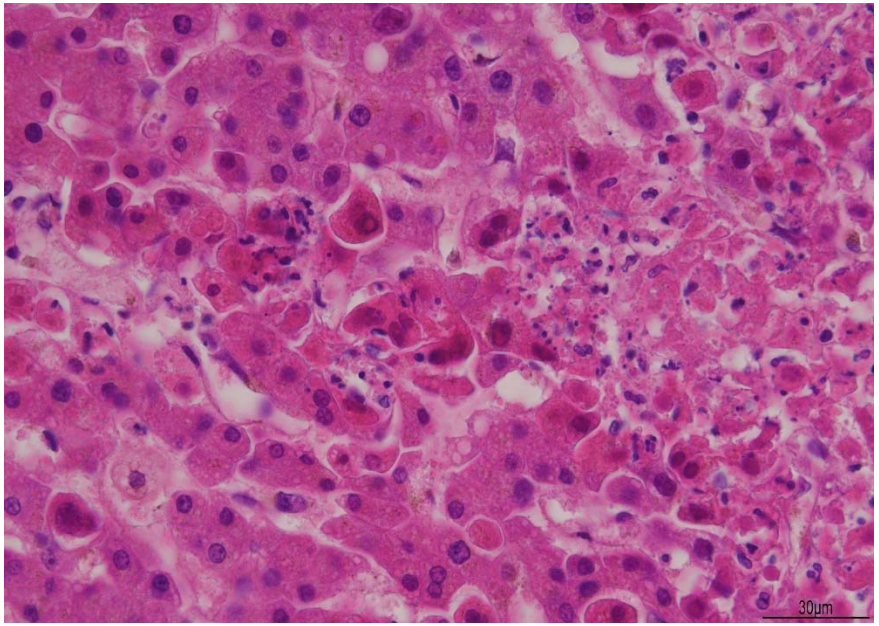

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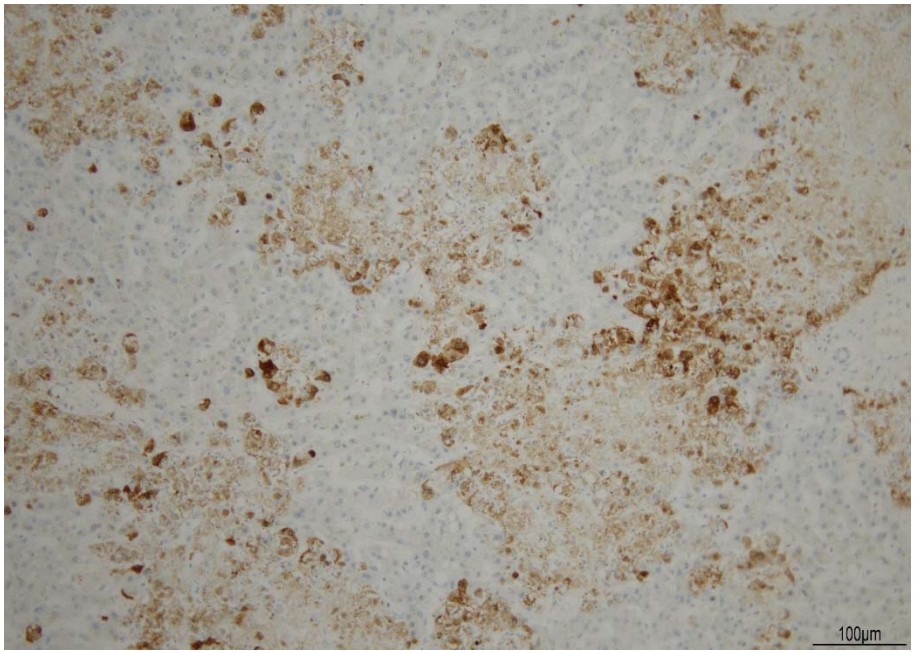

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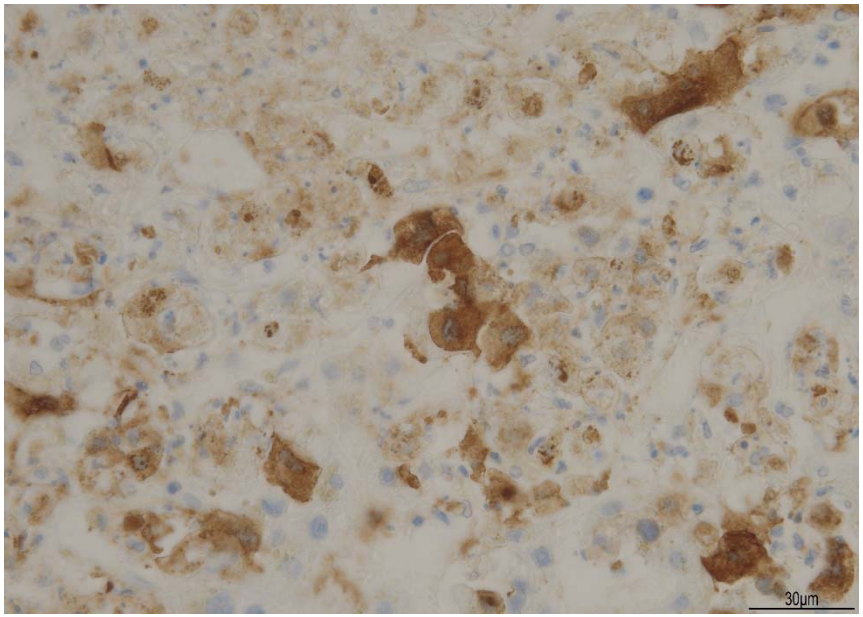
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Supplemental figure legend

Figure (a)(b)(c)(d): Immunohistochemical staining for cytomegalovirus.

Supplemental method: Nested PCR analysis for HHV-6.

DNA was extracted with QIAamp DNA FFPE Tissue Kit (QIAGEN) from two paraffin sections respectively. Nested PCR for HHV6 was performed with Prime STAR GXL DNA Polymerase (TAKARA Bio, Shiga, Japan) as described previously[1].

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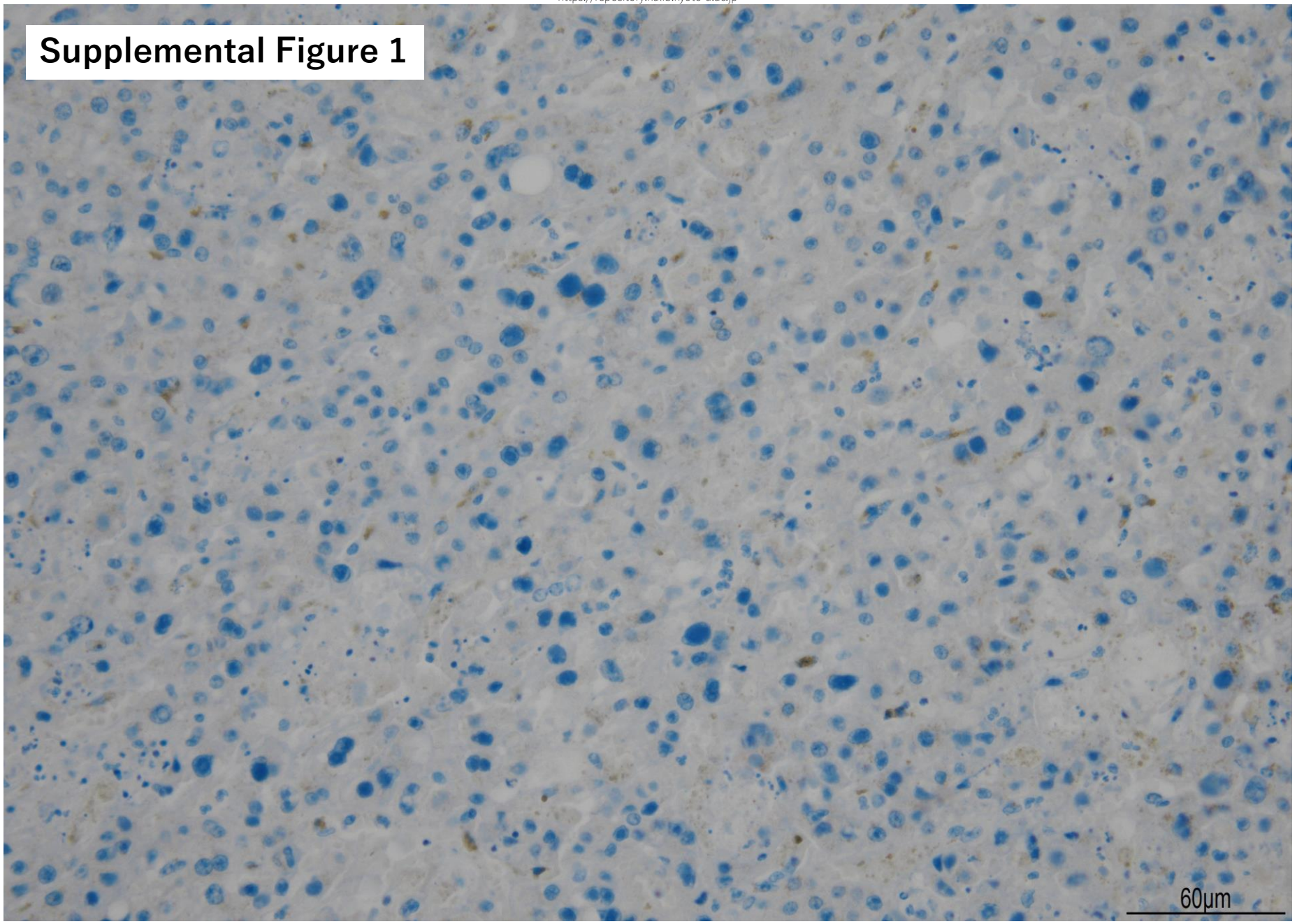
Supplemental Figure 1



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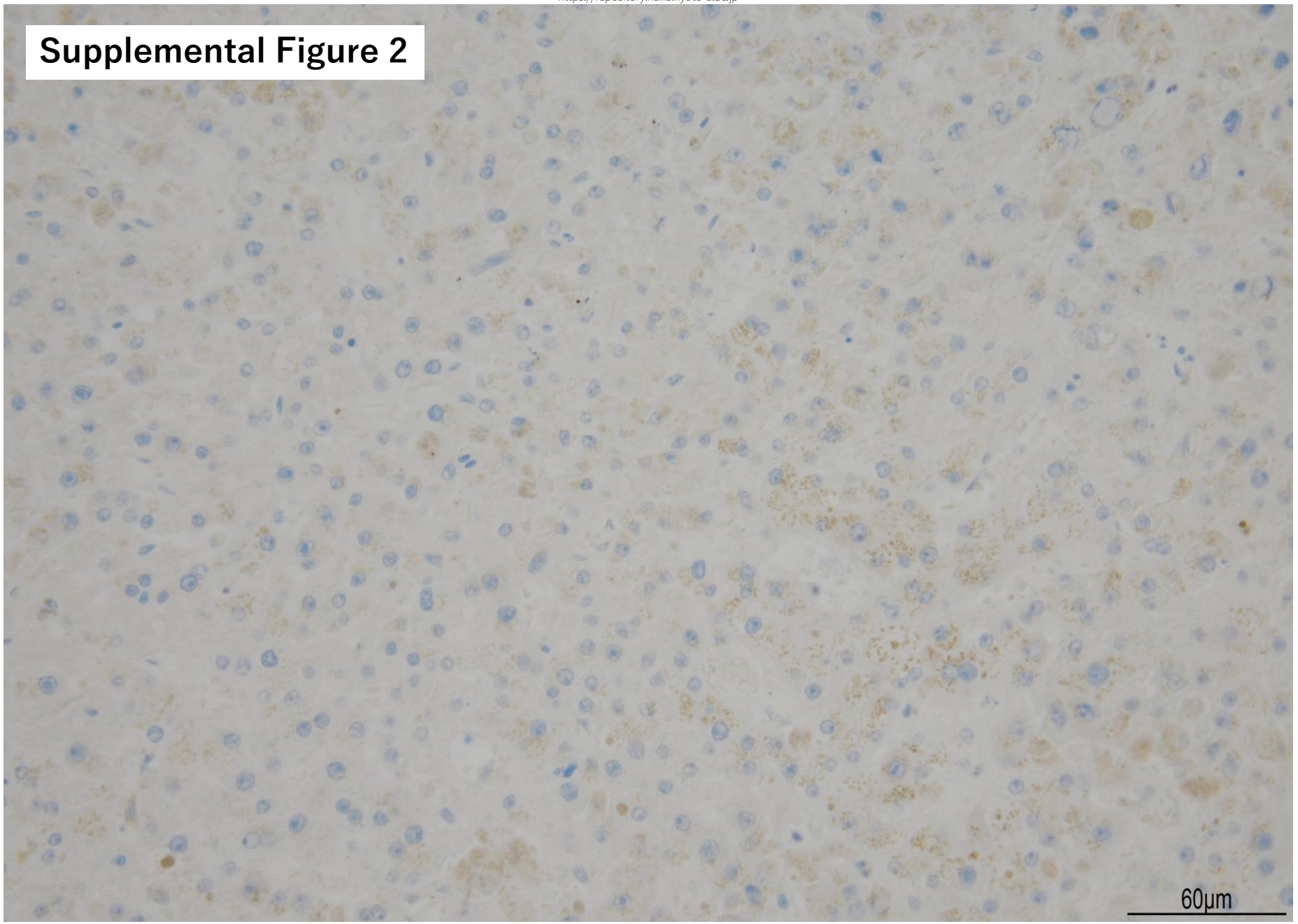
Supplemental Figure 2



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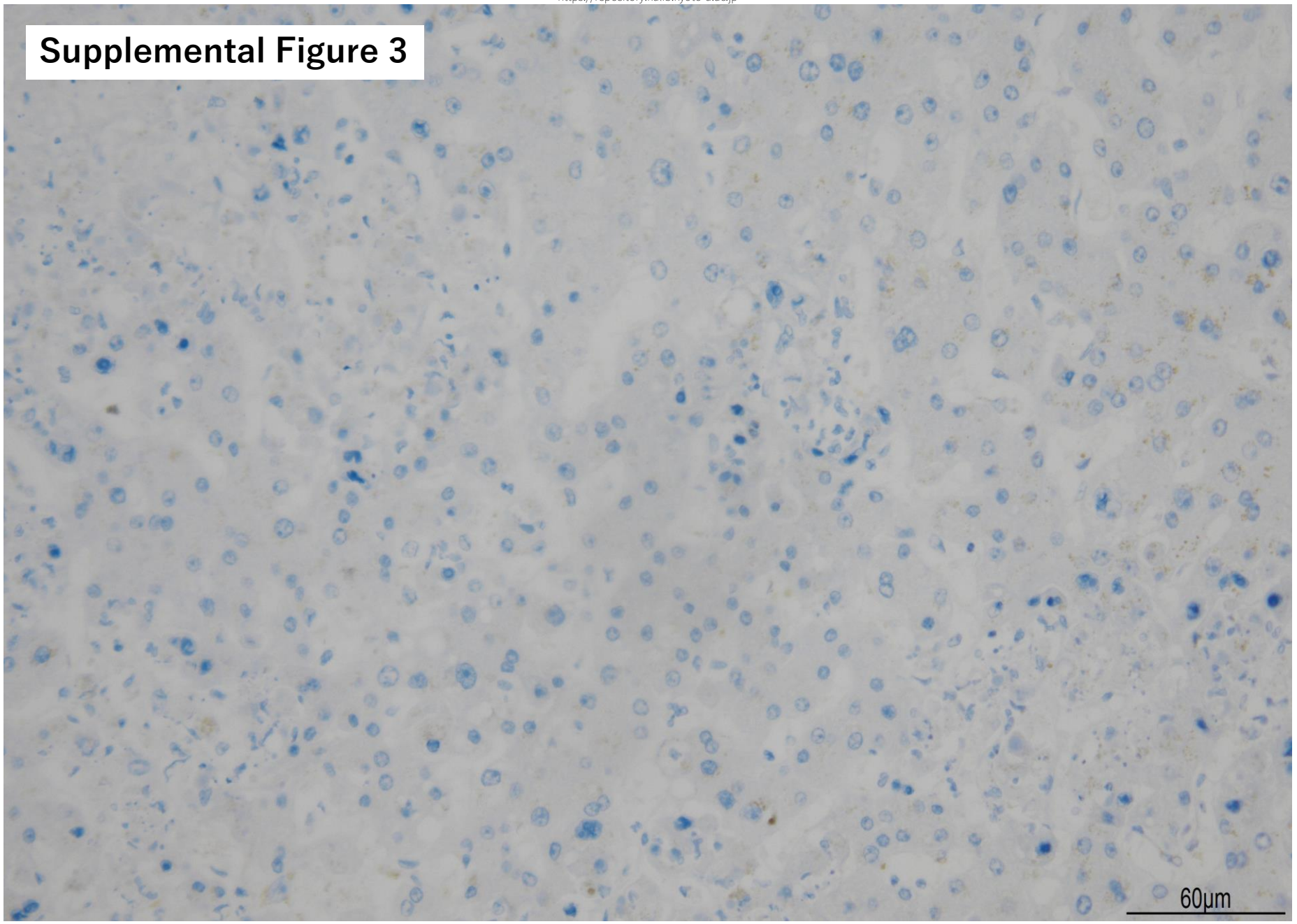
Supplemental Figure 3



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Supplemental Figure 4